^{99m}Tc-Imidodiphosphonate: a superior radiopharmaceutical for *in vivo* positive myocardial infarct imaging
I: *Experimental data*

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SUMMARY ^{99m}Tc-Imidodiphosphonate (^{99m}Tc-IDP) was investigated as an agent for nuclear medicine imaging of acute myocardial infarctions. For this purpose a suitable animal model was found. After coronary artery ligation reproducible myocardial infarcts were obtained with 80 per cent of surviving animals. Scans of the myocardial infarcts were recorded with a high resolution gamma camera and good quality images were obtained. ^{99m}Tc-IDP ratios for normal and infarcted tissue were calculated and compared with similar data available from other ^{99m}Tc-labelled phosphates. With an infarct/normal ratio of 21:1, ^{99m}Tc-IDP is so far the best radiopharmaceutical for nuclear medicine imaging of necrosed heart muscle. Images of the myocardial infarcts have been recorded as early as 6 hours after infarction.

Since it was first suggested that ^{99m}Tc-labelled phosphates may concentrate in acutely damaged myocardium (Bonte *et al.*, 1974) experience in this field has accumulated and a wealth of data has been produced. For the first time non-invasive imaging methods are available for the direct visualisation of acute myocardial infarction. The possibility of early diagnosis, sizing of the infarcted area, follow-up with time, and monitoring of the effect of treatment has attracted the clinician, the specialised cardiologist, and the physiologist interested in cardiac pathology. Two main approaches to acute myocardial infarct radionuclide imaging exist—the utilisation of potassium analogues, mainly ^{201}Thallium as thallous chloride and the utilisation of ^{99m}Tc-labelled radiopharmaceuticals, mainly as ^{99m}Tc-pyrophosphate.

The main advantage of ^{201}Thallium imaging within the context of acute myocardial infarction is the fact that accurate diagnosis can be made at a very early stage of the disease (Wackers *et al.*, 1976), that is 6 hours after the acute event. Important disadvantages, however, severely reduce the usefulness of this approach: the isotope is extremely expensive (in this country a single scan will cost between £30 and £50), serial imaging of the same patient is

undesirable because of cost, dosimetry, and long biological half-life characteristics, at least one-third of subendocardial lesions are not visualised with this technique (Pitt and Strauss, 1976), and negative rather than positive images of the infarcted area are obtained with additional difficulties for interpretation. An old infarct cannot be distinguished from a recent one and exact discrimination between acute infarction and ischaemia cannot be made (Wackers *et al.*, 1975).

The advantages of ^{99m}Tc-labelled pyrophosphate imaging within the context of acute myocardial infarction are: accurate diagnosis can be made 24 hours after the acute event with a false negative rate of less than 4 per cent (Parkey *et al.*, 1977), serial imaging of the same patient is eminently possible (the radiopharmaceutical is very cheap and can be 'home-made', and the dosimetry and biological half-life characteristics allow for daily images), subendocardial infarcts can be diagnosed though small lesions less than 3 g can be missed, and positive rather than indirect images of the acute infarction are recorded. An old infarct can be distinguished from a recent one since only the latter will concentrate these radiopharmaceuticals.

In Table 1 we show some of the physical and biological characteristics of the mentioned radio-

Table 1   Main radionuclides used for myocardial imaging purposes

Isotope	Ey (Kev)	T ½ hours	Radiation whole body (mRad/mCi)
K-43	373	22	700
Rb-81	511	4·7	100
Th-201	81	74	70
Tc-99m	140	6	15

pharmaceuticals. A variety of ^{99m}Tc-labelled radiopharmaceuticals are now available for acute infarct imaging (Table 2). At the 1976 meeting of the American Society of Nuclear Medicine, the results obtained in rabbits with experimentally induced myocardial infarction describing the uptake of six different ^{99m}Tc-labelled radiopharmaceuticals were reported (Grossman et al., 1976). Three ^{99m}Tc complexes (pyrophosphate, methylenediphosphonate, and imidodiphosphonate) produced infarct to myocardial ratios, at one hour after the intravenous administration of the radiopharmaceutical, of 5, 5, and 14. Previously it was shown (Subramanian et al., 1975) that imidodiphosphonate, an analogue of pyrophosphate and diphosphonate with P-N-P bond instead of P-O-P or P-C-P linkages showed considerably higher bone uptake than the previous agents, in fact similar to those obtained with ^{18}fluorine uptake in bone.

These findings prompted us to investigate the role of this new agent—^{99m}Tc-imidodiphosphonate —(^{99m}Tc-IDP). Our experience of this agent in whole body imaging of the skeleton has been the subject of a recent study (Ell et al., 1977). The purpose of this investigation was to identify the following problems:

(1) Is ^{99m}Tc-IDP a suitable agent for in vivo visualisation of acute myocardial infarction in humans ?

(2) Is this agent for this purpose superior to the most widely used ^{99m}Tc-pyrophosphate ?

(3) What is the relation of uptake of this radiopharmaceutical with the histological changes occurring in infarctions induced in an acceptable animal model ?

(4) Is myocardial infarct imaging helpful in the management of patients within the environment of our coronary care unit ?

Table 2 ^{99m} Tc-labelled pharmaceuticals in use for positive myocardial infarct imaging

^{99m}Tc-Tetracycline
^{99m}Tc-Glucoheptonate
^{99m}Tc-Dimercapto-succinic-acid
^{99m}Tc-Pyrophosphate
^{99m}Tc-Ethyl-hydroxy-diphosphonate
^{99m}Tc-Methylenediphosphonate
^{99m}Tc-Imidodiphosphonate

Materials and methods

Ten Wistar rats weighing between 200 and 300 g were submitted to a thoracotomy and ligation of the left coronary artery. A group of only 5 rats, acting as controls, underwent thoracotomy only.

The full surgical procedure involves removing the fur from the chest which is swabbed with spirit and a 2 ½ cm left parasternal skin incision is made under ether anaesthesia. The pectoralis major muscle is transected and retracted laterally exposing the rib cage. A cutaneo-muscular suture is inserted at the level of the 5th rib cage and arranged superiorly loosely knotted enabling the wound to be closed quickly at the end of the procedure. The point of a pair of fine scissors is inserted into the intercostal muscles below the 4th rib 2 to 3 mm lateral to the left sternal edge, so avoiding the internal thoracic artery. The muscles are split forming a small thoracotomy which is extended laterally along the intercostal space. Great care is taken to ensure that the ribs are not traumatised by the procedure. The pericardium is opened and by exerting light pressure on the back the heart may be exteriorised; the occluding stitch is tied around the left coronary artery close to its origin. The left coronary artery runs deep in the myocardium between the main pulmonary artery and the left auricular appendage so that a stitch placed between the two ligates the artery. Immediately after the ligation the heart is replaced in the thorax and the cutaneo-muscular stitch is pulled out while the thorax is lightly squeezed to reduce the residual pneumothorax. Michel clips are placed along the wound which is sprayed with antibiotics and plastic skin. The rats will resume breathing provided that the duration of thoracotomy is less than 90 seconds. Obstructing mucus is aspirated and a course of intramuscular antibiotics is begun. The rat is moving voluntarily by 15 minutes and eating and drinking by the second postoperative hour.

The labelling technique of the radiopharmaceutical is identical to the procedure described (Brody et al., 1976). Kits are made up sterile and pyrogen free ready for a single step labelling procedure and intravenous injection. Each kit contains the equivalent of 2·2 mg imidodiphosphoric acid and 0·26 mg tin. At room temperature 1 mCi of ^{99m}Tc pertechnetate in a small volume is added to the vial, the average labelling efficiency being about 95 to 97 per cent.

Imaging of these animals was performed one hour after injection of 1 mCi of ^{99m}Tc-IDP via the rat tail vein. Animal imaging was done on a Nuclear Enterprises Mk. V HR standard field high resolution gamma camera on line to a Varian 620 i computer

system. Images in the anterior and lateral projections were obtained 24 hours after induction of myocardial infarction. In addition, 3 animals were sacrificed at 6, 48, and 72 hours after induction of infarction in order to relate the histological appearance of the uptake pattern with tissue after infarction.

Each image was analysed for the presence of abnormal uptake of ^{99m}Tc-IDP over the left praecordium and a comparison was made between the infarcted and the control group of animals.

When imaging was completed the chest of the rats was reopened and the hearts inspected for evidence of infarction. The heart was then cut into slices and alternate slices were selected for histology and isotope counting respectively (the apex being used for both). The pieces of tissue derived from the sectioned heart were preserved for histological examination by fixation in 10 per cent formal saline. They were progressively dehydrated in a series of ethanol solutions and then embedded in paraffin wax after immersion in toluine. These so-formed blocks were cut into sections with a microtome and the tissue was immersed in xylol to dissolve out the wax. The sections were rehydrated for staining with haematoxylin eosin and phosphotungstic acid haemotoxylin. After staining, the sections were dehydrated, cleared, and mounted in dammar resin.

The slices for scintillation counting were divided into 15 small pieces and placed into preweighed numbered tubes. The position of each piece within the slice was recorded on a diagram which included the macroscopical appearance of the slice, with any area of infarction drawn on it. The tubes were weighed and counted in a well scintillation counter enabling the specific activities to be calculated for that type or position of myocardial biopsy. The lowest value of the intraventricular septal biopsy was taken as the unit value so that normalised figures for each slice were calculated. Any correlation between the region of the infarction confirmed by the histological examination of the adjacent slices and the distribution of the radiopharmaceutical was noted.

Results

In Fig. 1 a diagram of a slice of a heart of one of the animals without myocardial infarction is shown. The lowest count rate of the radiopharmaceutical in this slice was identified as unity, all other count rates found in this slice of the heart being normalised to this value. The lowest count rates in the control group of animals were usually found in the septal area of the myocardium, the variation between the lowest and the highest count rate value for all the animals of the control group being 0·8 to 2·5.
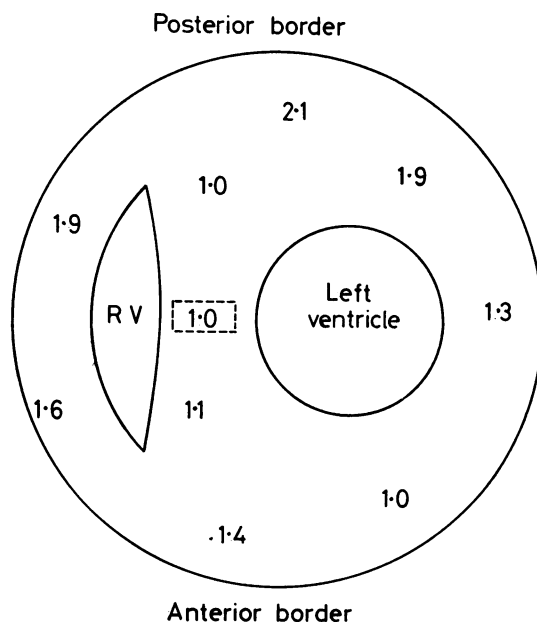

Fig. 1 *Distribution of ^{99m}Technetium-labelled IDP within the myocardium of a normal heart. Normalised values in a transverse slice of the ventricles.*
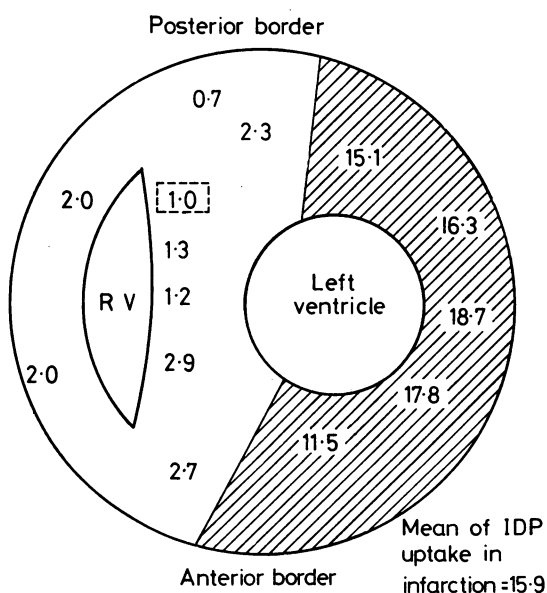
Fig. 2 *Distribution of ^{99m}Technetium-labelled IDP within the myocardium. Normalised values in a transverse slice of the ventricles of a heart with a surgically induced infarction.*
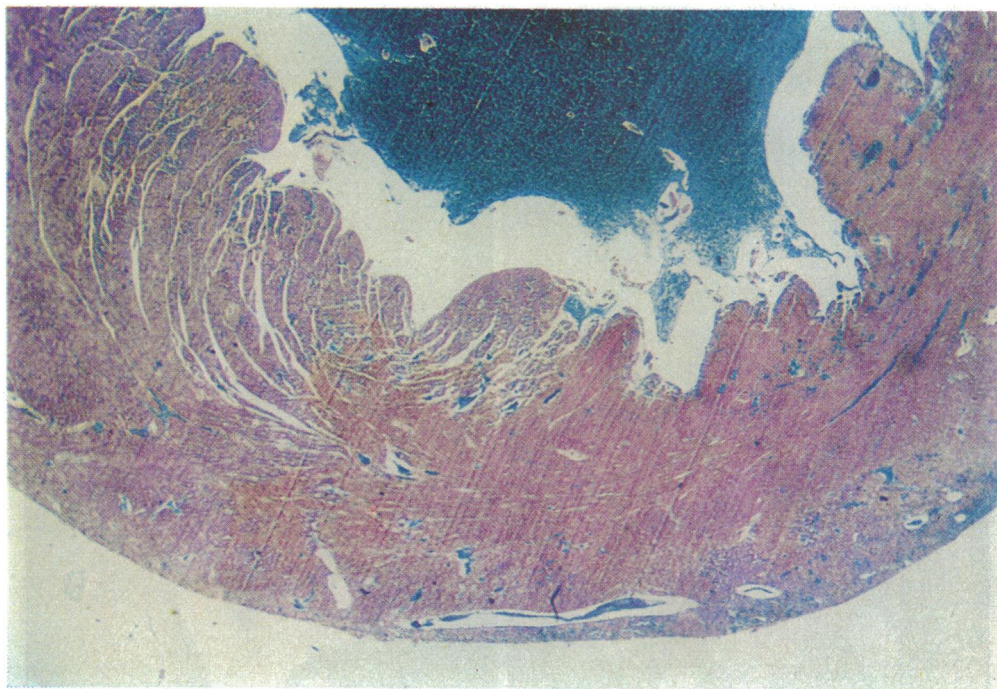
Fig. 3   *Micrograph from the heart of a rat that underwent the coronary ligation technique shows a well-demarcated left ventricular infarction. The purple PTAH staining of the normal myocardium is replaced on the right by a poorly stained (reddish) region.*
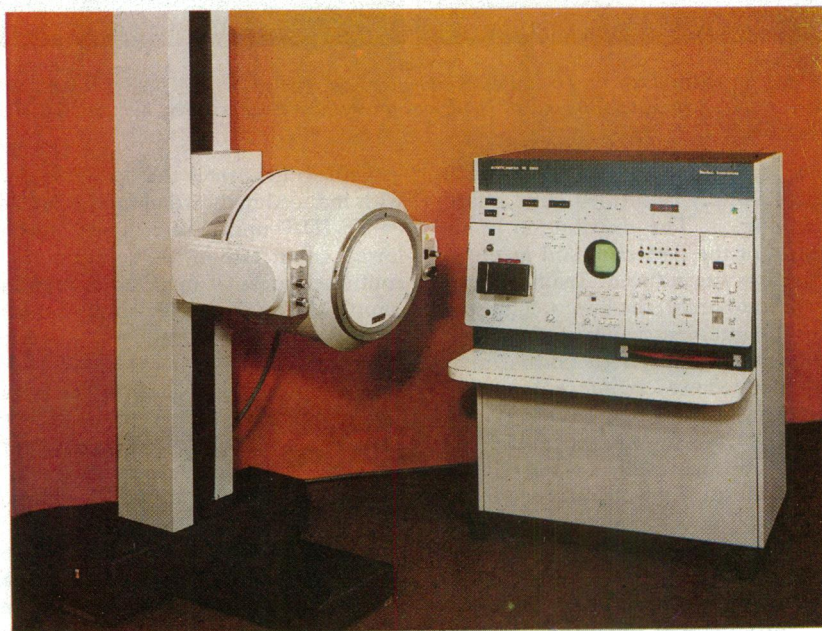

Fig. 4   *The Nuclear Enterprises MKV High Resolution gamma camera used in this investigation.*
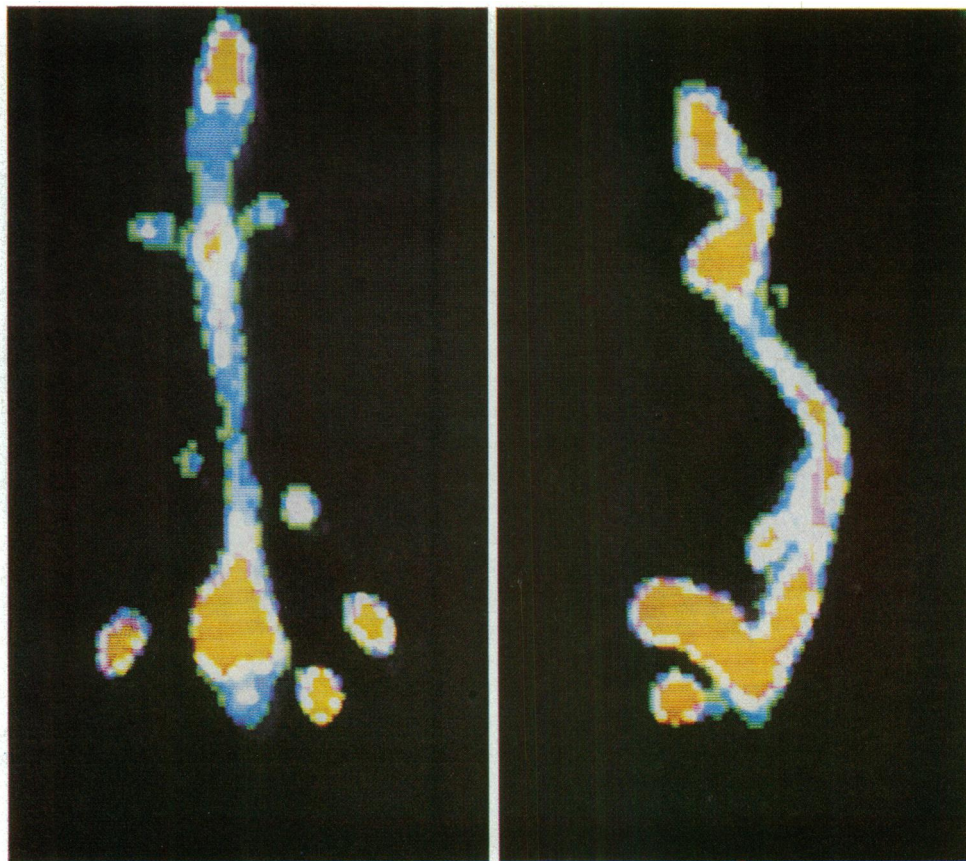
Fig. 5 and 6   *Control rat. ^{99m}Tc-IDP image 1 hour after injection. Lateral projections and antero-posterior. Note high uptake in the skeleton, no uptake over praecordium, kidney, and bladder visualisation.*

Fig. 2 is a typical diagram of a slice of a heart of an animal with a histologically confirmed large infarction illustrated in Fig. 3. The distribution of the count rate is typical of the pattern found in the 10 rats in which infarction was induced. Again all count rates were normalised to unity, the lowest count rate was also found in the interventricular septal region, the ratio of the count rates between normal myocardial tissue and infarcted myocardium being 1:18·7 in the example shown.

Fig. 4, 5, 6, 7, and 8 show the equipment used for imaging the animals and the result of some of these tests. Scans of excellent quality were obtained for all animals with good bone uptake, showing the excellent qualities of this radiopharmaceutical as a bone seeking agent, with no ^{99m}Tc-IDP uptake in the heart of the animals belonging to the control group (Fig. 5 and 6) and clear and intense ^{99m}Tc-IDP uptake in animals with induced acute myocardial necrosis (Fig. 7 and 8).

Table 3 illustrates the data obtained with 10 rats with induced myocardial infarction. Mean values of ^{99m}Tc-IDP uptake are given normalised to the lowest count rate in the septum, for histologically confirmed areas of myocardial infarction, for tissue samples taken from the centre of the infarction, and for the uptake of the radiopharmaceutical in normal bone (anterior rib).

A significant difference of the uptake pattern of ^{99m}Tc-IDP was, therefore, seen between normal heart tissue and infarcted myocardium. The mean ratio of activity between infarcted and normal ventricular myocardium in this series of animals was 21·6.

Images of myocardial infarction obtained with Tc-99m labelled phosphates are usually graded in four categories according to the intensity of uptake of the radiopharmaceutical over the chest: 4+ positive images are those where uptake in the heart is more intense than in the neighbouring bony
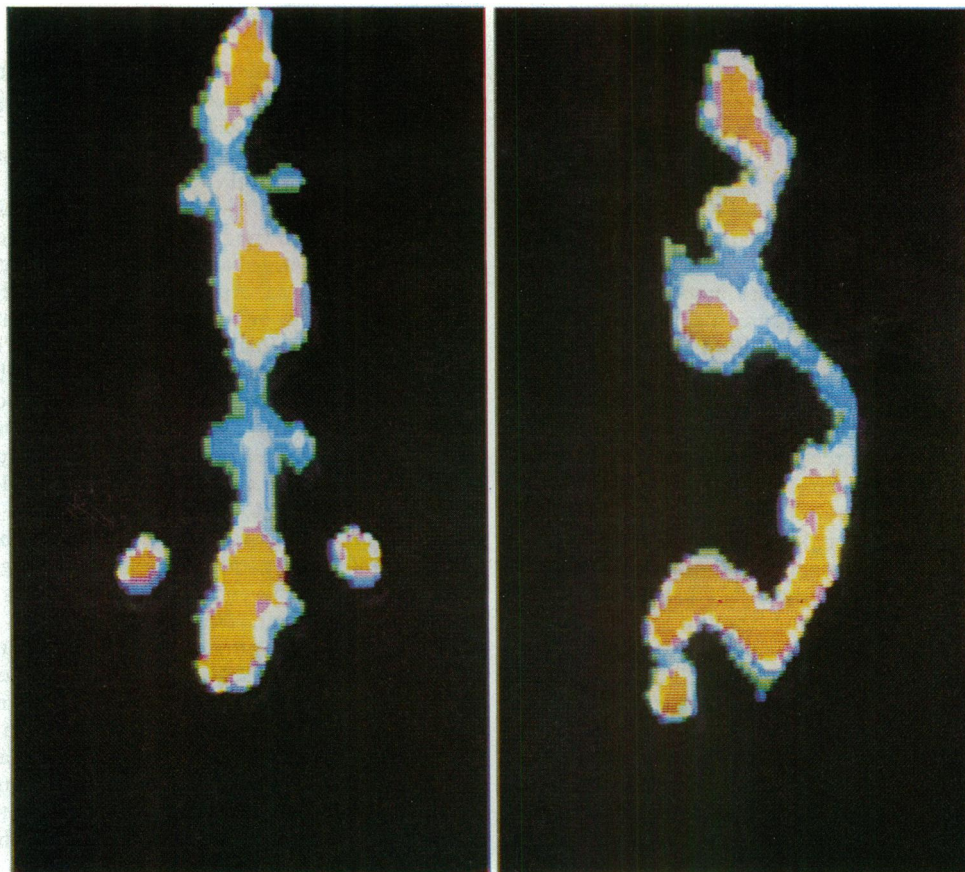
Fig. 7 and 8   Infarcted rat. ^{99m}Tc-IDP image 1 hour after injection. Lateral and anteroposterior projections. Note high uptake in skeleton and in the infarcted heart well seen in both projections.

structures (sternum and ribs); 3+ positive images are those where uptake is equal to the uptake observed in sternum or ribs; 2+ positive images are those where uptake of the radiopharmaceutical in the myocardium is less than uptake by bone but where the outline of the damaged tissue in the heart is still clearly seen; finally, 1+ positive images are those where the activity over the heart cannot be distinguished from general tissue and blood background radioactivity.

Using this criteria of classification all 10 animals where infarction was artifically induced presented with 3+ or 4+ positive images. Histologically the areas of highest ^{99m}Tc-IDP uptake correspond to distinct and severe cellular damage of the myocardium. In fact both the well scintillation counter and the gamma camera scans produced data that correlated extremely well with the histological

changes observed under microscope analysis.

The animals which had been subjected only to thoracotomy illustrated the pathological changes caused by the opening of the pericardium and

Table 3   Distribution of ^{99m}Tc-IDP in infarcted rats

IDP uptake in infarcted myocardium	IDP uptake at centre of infarction	IDP uptake in anterior rib
14·1	13·6	26·8
12·3	21·3	11·8
30·1	19·7	21·9
16·0	18·9	8·3
17·1	27·0	8·4
14·1	33·1	13·8
15·9	18·7	4·2
18·3	17·3	17·1
51·7	23·6	8·4
26·5	22·8	11·3
Mean		
21·6	21·6	13·2

manipulation of the heart. At necropsy examination there was the expected pericarditis confirmed by microscopy which also showed some patchy subpericardial necrosis. Phosphotungstic acid haematoxylin (PTAH) stained sections showed areas of myofibrillar swelling with loss of cross striations and disruption of muscle fibres, but there was no evidence of loss of fibres. The PTAH staining was uniform and purple throughout the sections. There was, in addition, patchy subendocardial damage but the sections did not indicate any of the regional changes of myocardial infarction attributable to the disruption of the coronary arterial blood supply. Examination of those animals which had undergone thoracotomy and left coronary artery ligation showed, apart from the already described pathological changes, a pale anterolateral region on the left ventricular myocardium corresponding to the territory supplied by the descending branch of the left coronary artery. After the arterial ligation, the pattern of histological changes developed so that 6 hours after operation the anterolateral and anterior septal left ventricular myocardium showed loss of staining with PTAH and the more reddish hue characteristically seen in infarcted tissue. There was great swelling of muscle fibres, vacuolation, and loss of striation. There were wavy fibres as described by Bouchardy and Majno (1974) at the margins of this damaged region. The remaining myocardium resembled that of the control rats which had undergone thoracotomy only. There was, therefore, a clearly visible almost transmural regional change in the left ventricular myocardium showing the early evolution of the pattern after coronary artery ligation and resultant ischaemic necrosis. At 24 hours there was in the anterolateral and the anteroseptal left ventricular wall pronounced loss of PTAH with a lighter reddish appearance. There was distinct loss of striation, break-up of some of the fibres, and myofibrillar swelling. There was a cellular infiltration related to the pericarditis and a mild eosinophilic infiltration. These sections disclosed a predominantly transmural myocardial infarction with a varied degree of survival of fibres immediately below the endocardium. The appearance at 48 hours was similar now with progressive loss of fibres and break-up of many fibrils. By 72 hours there was pronounced loss of fibres in the anterolateral and anterior myocardium of the left ventricle, with large areas of coagulative necrosis and replacement by granulation tissue with much mitotic activity. There was a brisk inflammatory reaction with an infiltration of predominantly mononuclear phagocytes. There was telangiectasia at the margin of the region of infarction and within the granulation tissue.

Discussion

In order to evaluate any new radiopharmaceutical as a potential tracer for acute myocardial imaging a suitable animal model will have to be found. Ideal models are not available. In fact, it is impossible to mimic in an animal the gradual time and tissue dependant changes of the coronary arterial circulation which occur in the human heart and which will lead to acute myocardial necrosis. Known clinical conditions such as unstable angina pectoris, Prinzmetal angina, or preinfarction angina are not reproducible in animal experimentation. With all these reservations it is nevertheless possible to induce experimentally in animals acute myocardial necrosis. There are two main models available depending on whether or not open chest surgery is performed. In earlier work a closed chest model was used (Grossman et al., 1976) which consisted in the blind introduction of a needle from below the diaphragm, injecting damaging and necrosing substances into the left ventricular wall, such as vasopressin in peanut oil. The method however is not easily reproducible, proving successful in only 50 per cent of the attempts. More important, however, is that fact that the necrosing and vasoconstricting effect of the vasopressin in peanut oil injection is not permanent. In fact, the infarcted area is available to reperfusion at an early stage. This phenomenon introduces a serious and unknown variable to any attempt to correlate histological tissue changes within and around the induced infarcted area of the myocardium with the distribution and mechanism of uptake of radiopharmaceuticals.

It was, therefore, decided to adopt the open chest surgical procedure described by Bajusz (1967) to induce an acute infarction in the animals undergoing the experiments. The method proved to be highly reliable, reproducible, and with a high recovery rate of the animals submitted to operation. Permanent occlusion of the coronary artery was obtained producing infarction, with 80 per cent of surviving animals. Furthermore, the irreversibility of the occlusion mimics to a certain extent the acute coronary occlusive episode and is not accessible to early reperfusion.

The data clearly indicate the advantage of using ^{99m}Tc-IDP as an imaging agent for acute myocardial infarction. The LD-50 of this substance in rabbits is of the order of 45-50 mg/kg as acid, allowing for a safety factor of at least 5000 in humans (Subramanian et al., 1975). In a period of 6 months approximately 600 human whole body bone scans were performed in this Department of Nuclear Medicine with ^{99m}Tc-IDP. Excellent whole body images were obtained and no adverse reaction to the radio-

pharmaceutical was noted. This agent has in fact become the departmental routine bone scanning agent.

The material is easy to prepare in the laboratory with standard sterile techniques. It is easily and conveniently labelled with ^{99m}Tc-pertechnetate, the standard labelling isotope. The cost of the material in its final form is very low (50p per patient/dose). The signal to noise ratio for this new radiopharmaceutical (infarcted myocardium/healthy myocardium = 21·6) is significantly better than those obtained either with the standard tracer for positive myocardial infarct imaging (for ^{99m}Tc-pyrophosphate it is 5:1) or other ^{99m}Tc-labelled phosphates used for the same purpose (for ^{99m}Tc-ethylhydroxidiphosphonate it is 9:1 and for ^{99m}Tc-methylenediphosphonate it is 5:1).

The correlation between the distribution of ^{99m}Tc-IDP in the heart of the animals with induced acute myocardial necrosis and the histological changes was revealing. Only in those areas where necrotic damage of myocardial tissue was present was intense uptake of ^{99m}Tc-IDP found. None of the animals from the control gave a 'false-positive' image and all animals submitted to myocardial infarction gave clear-cut 3 or 4 plus positive images, with no 'false-negative' images. In addition positive images were obtained as early as 6 hours after infarct induction and certainly were present at 24 hours and 48 hours.

Conclusion

^{99m}Tc-IDP is the ^{99m}Tc-labelled phosphate of choice for acute myocardial imaging. As early as 6 hours after infarct induction in rats it offers high quality images of the infarcted hearts and at a very low cost. Because of very favourable dosimetry (15 mRad/mCi) serial imaging of patients can be considered. Therefore, a reliable imaging technique can be offered allowing for positive diagnosis, sizing, and quantification of acute myocardial infarction.

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